Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

1. General information and methods

Materials.

Unless otherwise noted, all reagents were obtained from commercial resources and used without further purification. Tetrahydrofuran (THF), toluene (Tol), and dichloromethane (DCM) used for reactions were purified by evaporating after fully stirring with Na and using benzophenone as the indicator. All air and moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere.

Preparation of surfactant-chaperoned S-D-A-D-S dye.

Triton-x100 was dissolved as a 30% (w/w) solution with DI Water and incubated for one hour at 50°C in an oven. Then, the solution was further diluted to 5% in (w/w) PBS as a stocker. 20 μL of stocker and 100 μL IR-FH4P (2 mM in DMSO) were added to PBS to form a 10 mL system and mixed by the vortex. After incubating in an oven at 50°C for one hour, the reaction system was stored at room temperature for one hour in the dark. IR-FH4P/Triton solution was finally concentrated to 1 mL by centrifugation at 10000 RPM with ultrafiltration centrifuge tubes (~3000 M.w.). The product was stored at 4°C away from light for further use.

Measurements.

All ^{1}H-NMR spectra were obtained on Bruker AVANCE III 500 MHz or 400 MHz NMR spectrometers (Q. One Instruments Ltd.). All compounds were subjected to ^{1}H-NMR analysis to confirm ≥ 95% sample purity. Chemical shifts were reported in ppm relative to the residual solvent peak ($CDCl_3$: ^{1}H, 7.26) or tetramethylsilane (TMS) peak. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), m (multiple), dd (doublet of doublets). All coupling constants (*J*) are reported in Hertz (Hz). Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) was performed on a Bruker Microflex TOF using trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-2propenylidene] malononitrile (DCTB) as a matrix. Ultraviolet-visible-near infrared (UV-VIS-NIR) absorption spectra were recorded on PerkinElmer Lambda 950. Fluorescent emission spectra were tested on Edinburgh Instruments FL 920. Size exclusion chromatography (SEC) was performed on Waters. 1515.

Measurement of (high-resolution mass spectrum) HRMs.

The mass spectrometry was operated under the specific conditions (ESI+ spray voltage, 4.5 Kv, or ESI- spray voltage, -3.5 Kv; nebulizer gas, 1.5 L/min; drying gas, 100 kpa; heat block temperature, 200℃; CDL temperature, 200℃; IT Area Vacuum, 1.0×10-2 Pa; TOF Area Vacuum, 5×10-4 Pa). The ion accumulation time was set to 10 ms, and the detector voltage was fixed at 1.6 kV.

The mass number calibration (ion trap and TOF analyzer) was completed using a solution of trifluoroacetic acid (TFA) and sodium hydrate. Data acquisition and analysis were performed using the LCMS Solution version 3.0 software.

Quantum yield test.

To measure the fluorescence spectra in the region of 900-1500 nm, a spectrometer (Edinburgh Instruments FL 920) under Xe 900 was used. An InGaAs camera (Teledyne Princeton Instruments, NIRvana 640) was used to measure the fluorescence intensity in the region of 900-1700 nm including *in vivo* imaging under an 808 nm laser excitation (Changchun New Industries Optoelectronics Tech. Co. Ltd). One 850-nm short-pass (SP) filter (ThorLabs) was used as the excitation filter and a 900-nm long-pass (LP) filter (ThorLabs) was used as an emission filter. All samples were measured at 25 ℃, and reference fluorophore, high-pressure carbon monoxide-synthesized (HiPco) SWCNT was used with optical density (OD) less than 0.1 at 808 nm. The NIR-II fluorescence emission intensities were measured under the same 808 nm excitation. The QY values of samples were determined based on five concentrations with gradient ODs at 808 nm. Using the measured ODs at 808 nm and spectrally integrated fluorescence intensity, the quantum yield of a test sample can be calculated according to the following equation:[1]

$$\varphi_x(\gamma) = \varphi_{std}(\gamma) \times \frac{F_X}{F_{std}} \times \frac{A_{std}(\gamma)}{A_x(\gamma)} \times \left(\frac{\eta_x}{\eta_{std}}\right)^2$$

HiPco QYs correction

The fluorescence quantum yields of the fluorophores were measured using previously reported procedures with few modifications.[1] The fluorescence quantum yields were determined against the reference fluorophore HiPco SWCNT with a known quantum yield of 0.40%[2] in aqueous solutions. HiPco SWCNTs were used to measure quantum yield (QYs) under an 808 nm laser excitation. IR-26 (QY=0.05-0.5 % as reported[3]) was used to correct the QYs of our HiPco single-walled carbon nanotubes (SWCNTS) and the QYs of our homemade HiPco SWCNTs were calculated to be 0.06-0.5% (φ_{std}) (**Fig. S2**e-h).

Photostability.

IR-FH4P was dissolved in PBS, FBS, or blood from rats with an OD of 0.20 at 808 nm. The fluorescence signal was monitored using a two-dimensional InGaAs camera under continuous exposure to an 808-nm laser at a power density of 88 mW/cm^2. The average fluorescence intensity of the region of interest (ROI) was plotted as a function of time.

Stability of co-assembled nanoparticles.

To measure the stability of the IR-FH4P/Triton complex, 600 μL IR-FH4P/Triton (200 μM) was separated into 6 vials (100 μL each). 3 vials were stored at room temperature without light and the others are stored at 4°C in the refrigerator. The absorption spectra and FL Intensity were measured every two days.

Animal experiments.

All animal experiments were conducted under the institutional guidelines and were approved by the Experimental Animal Ethical Committee of Jilin University (Protocol number: 20210642). Balb/C mice were purchased from Liaoning Changsheng biotechnology co. Lt. Bedding, nesting materials, food, and water were provided *ad libitum*. Ambient temperature was controlled at 20 to 24 °C with 12-hour light/12-hour dark cycles.

NIR-II fluorescence imaging.

Under 808 nm excitation, 850 nm SP and 900/1100 nm LP were used to collect NIR-II imaging under the InGaAs camera. **IR-FH4P** suspended in PBS (100 μL, OD = 0.5 at 808 nm) was

intravenously injected into a six-weeks-old Balb/C mouse (n = 3). Video-rate imaging was carried out immediately after the injection to monitor the blood perfusion in real time for the first 10 minutes. The images were further obtained at 0 min, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h, and 144 h post-injection. For surfactant-chaperoned dye, **IR-FH4P** with SDS (100 μL dye, OD = 0.5 at 808 nm, 10 μM surfactant)**, IR-FH4P** with Triton-X100 (100 μL dye, OD = 0.5 at 808 nm, 10 μM surfactant) were applied.

Breast tumor NIR-II fluorescence imaging.

Under 808 nm excitation laser, 850 nm SP and 900/1000 nm LP were used to collect NIR-II bioimaging. **IR-FH4P** (100 μL, OD = 0.5 at 808 nm), **IR-FH4P** with Triton-X100 (100 μL dye, OD = 0.5 at 808 nm, 10 μM surfactant) suspended in PBS were injected intravenously into an eight-week-old Balb/C mouse (n = 2) with breast tumor inoculated by 4T1 cells. The NIR-II images were obtained at 0 min, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h, and 96 h post-injection.

Cell experiment.

The Raw 264.7, 4T1, and L-02 (also named LO2, or LO_2) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, gibco) supplemented with 10% FBS (*Yeasen* biotech Co., Ltd.), 100 U mL^{-1} penicillin, and 0.1 mg mL^{-1} streptomycin. All cells were cultured under 5% CO_2 at 37 °C in an incubator (Thermo).

In vitro fluorescence imaging.

The Raw 264.7 cells (1×10^6 per well) and 4T1 cells were seeded into a confocal cell culture dish (NEST) and incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. Then, the DMEM solution with 5 μM IR-FH4P or 5 μM IR-FH4P/Triton was added. After 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h of incubation, the cells were rinsed three times with PBS to remove excess dye and collected in a small tube, and fixed with paraformaldehyde fixative. The tubes were imaged by the InGaAs camera under continuous exposure to an 808-nm laser at a power density of 88 mW/cm^2 (850 nm SP and 900/1100 nm LP filters were used).

Cell viability assessment using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.

The 4T1 and LO_2 cells (1×10^4 per well) were seeded into a 96-well plate (NEST) and incubated for 12 h at 37 °C in a humidified incubator with 5% CO_2. Then, the DMEM solution with 0.1, 0.2, 1, 2, 10, and 20 μM IR-FH4P, IR-FH4P/Triton-X100, and pure Triton-X100 was added. After 24 h of incubation, the cells were rinsed three times with PBS and a 200 μL MTT (0.5 mg/mL) solution was added. After removing the MTT solution after 4 h of incubation, 200 μL DMSO was added to each well. Placing the shaking table at a low speed for 10 min made the crystal fully dissolve. The absorbance value of each well was measured at OD 490 nm by Elisa reader (BioTek Synergy LX).

Density functional theory calculations.[1]

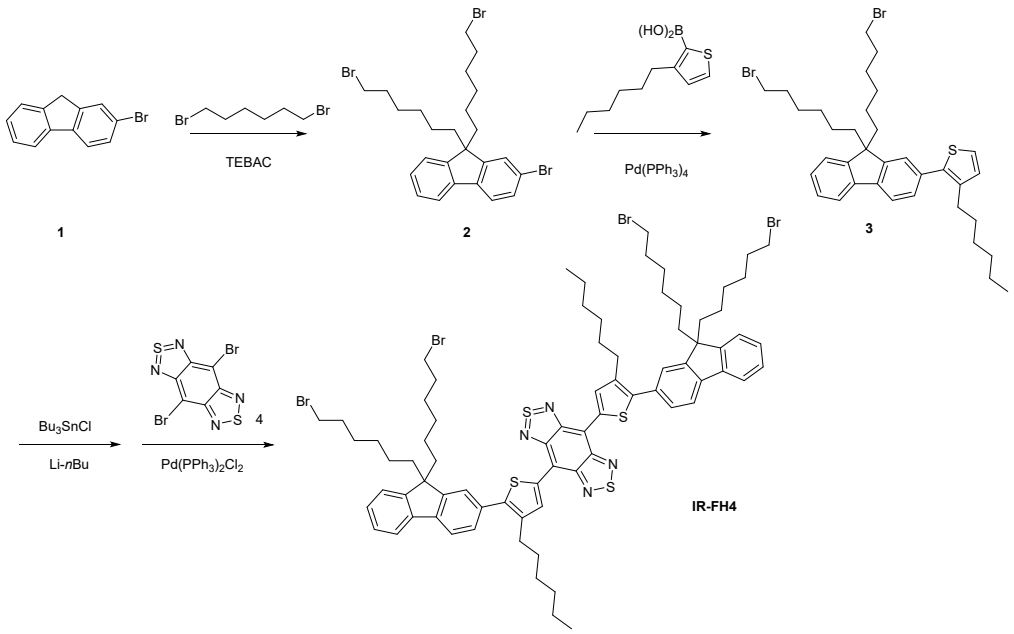
All the calculations were performed using the Gaussian 16 software in vacuum. To reduce the computational cost, alkyl substituent groups on fluorene units were replaced by methyl groups. The ground-state (S_0) geometries of the simplified structures **IR-FH4** and **IR-FH3** were firstly optimized using the *b31yp/6-31g(d)* method. The HOMOs and LUMOs, absorption energies of these molecules were obtained at the *b31yp/6-31g(d)* level based on their optimized S_0 geometries.

Statistical Analysis.

All FL Intensity data are expressed as mean ± SD (n=3 or 6 for biological experiment signal acquisition; n=6 for optical *in vitro* characterization). For normally distributed data sets with equal variances, one-way ANOVA testing followed by a Tukey post-hoc test was carried out across groups. In all cases, significance was defined as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Statistical analysis was carried out using OriginPro 2018 and Excel 2021.

2. Synthetic procedures and characterization data for the molecular fluorophores.

Scheme S1. Synthesis of IR-FH4.



1. **Synthesis of 2-Bromo-9,9-bis(6-bromohexyl)-9H-fluorene (2).** A portion of 1,6-dibromohexane (48.8 g, 200 mmol) was added into a mixture of 2-Bromo-9H-fluorene (2.45 g, 10.0 mmol) and benzyl triethylammonium chloride (50.0 mg) in 20 mL of dimethyl sulfoxide (DMSO) and 20 mL of 50 %wt aqueous sodium hydroxide (NaOH). The reaction mixture was stirred for 4 h at 80 °C. A portion of 50 mL of water was added to the reaction mixture, and then the reaction mixture was extracted three times with a portion of 50 mL of DCM. The combined organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed by using a rotary evaporator. 1,6-dibromohexane was recovered by vacuum distillation. The crude product was purified by column chromatography on silica gel using DCM/hexane. The yield of light-yellow liquid was 87.3% (4.8 g).

   ^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 7.68 – 7.65 (m, 1H), 7.57 – 7.55 (d, J = 10.00 Hz, 1H), 7.47 – 7.45 (m, 2H), 7.36 – 7.30 (m, 3H), 3.28 (t, J = 1.00 Hz, 4H), 2.00 – 1.89 (m, 4H), 1.68 – 1.63 (m, 4H), 1.22 – 1.16 (m, 4H), 1.12 – 1.03 (m, 4H), 0.65 – 0.55 (m, 4H).

^{13}C NMR (126 MHz, Chloroform-d) δ 152.37, 149.68, 140.32, 139.77, 129.83, 127.39, 126.88, 125.83, 122.58, 120.91, 120.85, 119.63, 55.02, 39.89, 33.71, 32.40, 28.78, 27.53, 23.72.

HRMS (ESI) *m*/*z*: calculated for $C_{25}H_{31}Br_3^+$ ([M] $^+$): 568.000. Found: 567.9970.

2. **Synthesis of 2-(9,9-bis(6-bromohexyl)-9H-fluorene-2-yl)-3-hexylthiophene (3).** 2-Bromo-9,9-bis(6-bromohexyl)-9H-fluorene (4.0 g, 7 mmol) and (3-hexylthiophen-2-yl) boronic acid (1.27 g, 6 mmol) were dissolved in mixture of THF (35 mL) and Na_2CO_3 solution (35 mL) under protective gas atmosphere into a flask bottle with $Pd(PPh_3)_4$ (300 mg, 0.26 mmol). After refluxing for 24 h and then cooling to room temperature, the mixture was extracted three times with 200 mL ethyl acetate and 200 mL brine. The organic phase was combined and dried with anhydrous $MgSO_4$ following by evaporating in vacuo. After purifying by silica column (Hexane/DCM 20:1), 3.51 g light yellow liquid was obtained as product **3** (88%).

^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 7.79 (t, J = 15.00 Hz, 2H), 7.51-7.49 (m, 2H), 7.43 – 7.36 (m, 3H), 7.29 (d, J = 5.00 Hz, 2H), 3.32 (t, J = 10.00 Hz, 4H), 2.78 (t, J = 10.00 Hz, 2H), 2.08 (t, J = 10.00 Hz, 4H), 1.75 – 1.72 (m, 4H), 1.42 – 1.34 (m, 6H), 1.30 – 1.24 (m, 4H), 1.17 – 1.12 (m, 4H), 0.96 – 0.93 (m, 2H), 0.83 – 0.70 (m, 4H).

^{13}C NMR (126 MHz, Chloroform-d) δ 152.38, 143.09, 142.16, 140.39, 140.28, 134.97, 131.54, 130.17, 129.12, 128.87, 125.49, 125.31, 124.66, 121.71, 121.62, 56.83, 42.12, 35.69, 34.51, 33.57, 32.98, 31.18, 30.97, 30.83, 29.79, 29.67, 25.50, 24.53, 15.17.

HRMS (ESI) *m*/*z*: calculated for $C_{35}H_{46}Br_2S^+$ ([M+H] $^+$):657.170. Found: 657.1760.

3. **Synthesis of IR-FH4.** To a solution of compound **3** (2.0 g, 3 mmol) in THF (50 mL) at -78 °C under N_2 protection, *n*-BuLi (1.6 M in hexane, 3 mL, 4.8 mmol) was added dropwise. After stirring at -78 °C for 1.5 h, tributyltin chloride (1.8 g, 5.5 mmol) was added to the solution. The reaction mixture was then slowly warmed to room temperature and stirred overnight. After that, the mixture was extracted three times with 100 mL ethyl acetate and 100 mL brine. The combined organic phase was dried with anhydrous $MgSO_4$ and evaporated in vacuo without further purification.

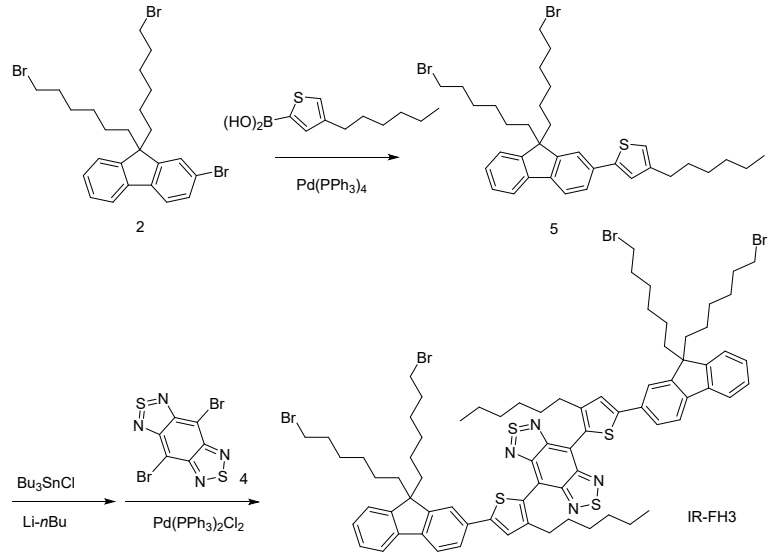
The crude product (3.5 g, 3 mmol) and compound **4** (350 mg, 1 mmol) were added in toluene (30 mL) under N_2 as protection gas atmosphere, and $Pd(PPh_3)_2Cl_2$ (200 mg) was added. After refluxing for 24 h, the mixture was cooled to room temperature. The mixture was extracted three times against 200 mL ethyl acetate and 200 mL brine. The organic phase was dried with anhydrous $MgSO_4$ and evaporated in vacuo. After silica column (Hexane/DCM 3:1) purification, **IR-FH4** was obtained as a dark green solid (454 mg, 31%).

^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 7.73 – 7.71 (m, 6H), 7.41 (d, J = 5.00 Hz, 2H), 7.38 – 7.39 (m, 8H), 3.27 (t, J = 10.00 Hz, 8H), 2.69 (t, J = 10.00 Hz, 4H), 2.00-1.96 (m, 8H), 1.69 – 1.59 (m, 12H), 1.34 – 1.31 (m, 12H), 1.25 – 1.04 (m, 16H), 0.90 – 0.84 (m, 6H), 0.72 – 0.60 (m, 8H).

^{13}C NMR (126 MHz, Chloroform-d) δ 153.30, 147.69, 145.85, 141.14, 140.09, 134.97, 131.54, 130.17, 129.12, 128.87, 125.49, 125.31, 124.66, 121.71, 121.62, 55.12, 40.42, 33.99, 32.81, 31.86, 31.27, 29.47, 29.27, 29.12, 28.08, 27.96, 23.79, 22.83, 17.55, 14.32.

HRMS (ESI) *m*/*z*: calculated for $C_{76}H_{90}Br_4S_4N_4^+$ ($[M+H]^+$): 1503.2800. Found: 1503.2855.

Scheme S2. Synthesis of IR-FH3.



4. **Synthesis of 2-(9,9-bis(6-bromohexyl)-9H-fluorene-2-yl)-2-hexylthiophene (5).** 2-Bromo-9,9-bis(6-bromohexyl)-9H-fluorene (4.0 g, 7 mmol) and (2-hexylthiophen-2-yl) boronic acid (1.27 g, 6 mmol) were dissolved in toluene (70 mL) under N_2 as protection gas atmosphere into a flask bottle with $Pd(PPh_3)_4$ (300 mg, 0.26 mmol). After refluxing for 24 h and then cooling to room temperature, the mixture was extracted three times with 100 mL ethyl acetate and 100 mL brine. The organic phase was dried with anhydrous $MgSO_4$ and evaporated in vacuo. After silica column (Hexane/DCM 10:1) purification, a light yellow liquid was obtained as product **5** (3.51 g, 88%).

^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 7.75-7.72 (m, 2H), 7.65-7.63 (d, J = 10.00 Hz, 1H), 7.60 (s, 1H), 7.41 – 7.35 (m, 3H), 7.30 (d, J = 10.00 Hz, 2H), 3.31 (t, J = 10.00 Hz, 4H), 2.70 (t, J = 10.00 Hz, 2H), 1.77 – 1.68 (m, 4H), 1.46 – 1.39 (m, 8H), 1.25 – 1.21 (m, 4H), 1.16 – 1.09 (m, 4H), 0.76 – 0.88 (m, 2H), 0.76 – 0.65 (m, 4H).

^{13}C NMR (126 MHz, Chloroform-d) δ 151.40, 150.47, 145.12, 144.19, 142.41, 142.30, 136.99, 133.57, 132.20, 131.14, 130.89, 127.51, 127.33, 126.68, 123.73, 123.64, 58.85, 44.14, 37.71, 36.54, 35.59, 35.00, 33.20, 33.00, 32.85, 31.81, 31.69, 27.52, 26.55, 17.19.

HRMS (ESI) *m*/*z*: calculated for $C_{35}H_{46}Br_2S^+$ ($[M+H]^+$): 657.170. Found: 657.1760.

5. **Synthesis of IR-FH3.** In a solution of compound **5** (2.0 g, 3 mmol) in THF (50 mL) at -78 °C under N_2 protection, *n*-BuLi (1.6 M in hexane, 3 mL, 4.8 mmol) was added dropwise. After stirring at -78 °C for 1.5 h, tributyltin chloride (1.5 g, 4.6 mmol) was added to the solution. The reaction mixture was then slowly warmed to room temperature and stirred overnight. After that, the mixture was extracted three times with 100 mL ethyl acetate and 100 mL brine. The combined organic phase was dried with anhydrous $MgSO_4$ and evaporated in vacuo without further purification.

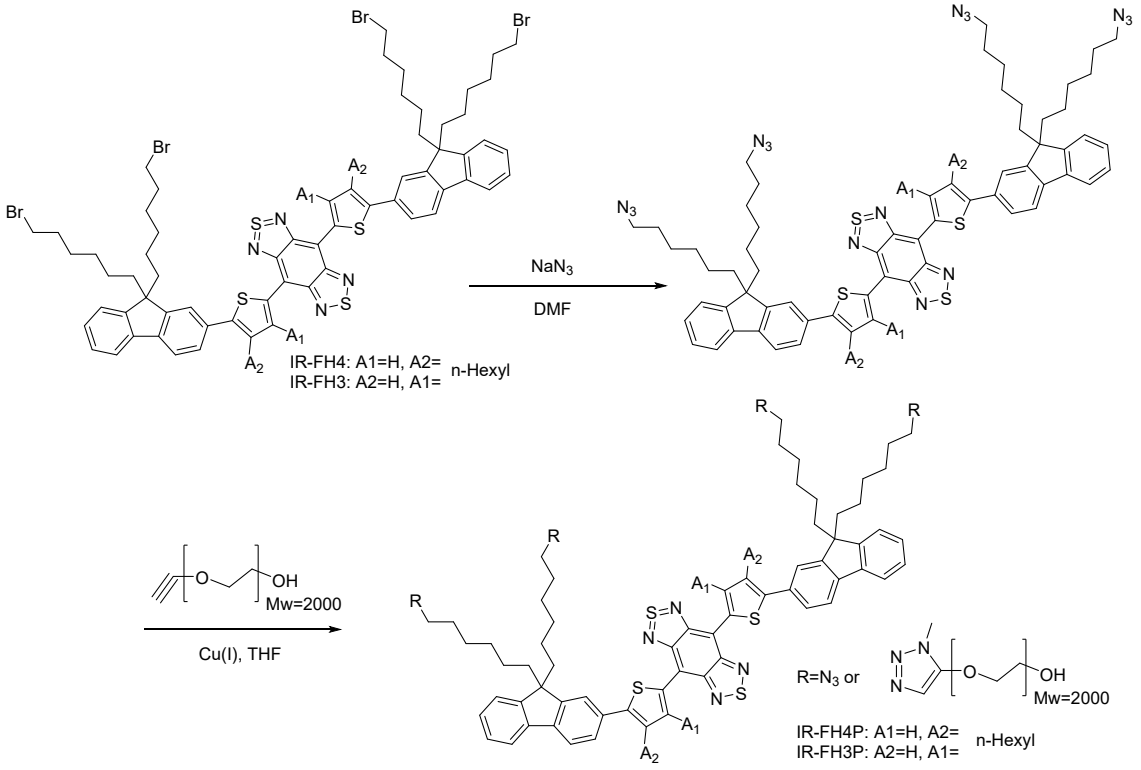
   The crude product (3.5 g, 3 mmol) and compound **4** (350 mg, 1 mmol) were added in toluene (30 mL) under protection gas atmosphere, $Pd(PPh_3)_2Cl_2$ (200 mg, 0.28 mmol) was added. After refluxing for 24 h, the mixture was cooled to room temperature. The mixture was extracted three times with 200 mL ethyl acetate and 200 mL brine. The organic phase was dried with anhydrous $MgSO_4$ and evaporated in vacuo. After silica column (Hexane/DCM 5:1) purification, **IR-FH3** was obtained as a dark green solid (454 mg, 31%).

   ^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 7.75 – 7.72 (m, 6H), 7.65 (s, 2H), 7.54 (s, 2H), 7.37 – 7.31 (m, 6H), 3.27 (t, J = 10.00 Hz, 8H), 2.64 (t, J = 10.00 Hz, 4H), 2.01-1.97 (m, 8H), 1.71 – 1.61 (m, 12H), 1.43 – 1.31 (m, 12H), 1.22 – 1.03 (m, 16H), 0.92 – 0.89 (m, 6H), 0.69 – 0.55 (m, 8H).

   ^{13}C NMR (126 MHz, Chloroform-d) δ 153.30, 151.12, 150.72, 147.69, 145.85, 141.14, 140.09, 133.10, 129.72, 128.89, 125.94, 125.85, 122.78, 119.40, 119.22, 118.57, 116.20, 55.12, 40.42, 33.99, 32.81, 31.86, 31.27, 29.47, 29.27, 29.12, 28.08, 27.96, 23.79, 22.83, 17.55, 14.32.

   HRMS (ESI) *m*/*z*: calculated for $C_{76}H_{90}Br_4S_4N_4^+$ ($[M+H]^+$): 1503.2800. Found: 1503.2855.

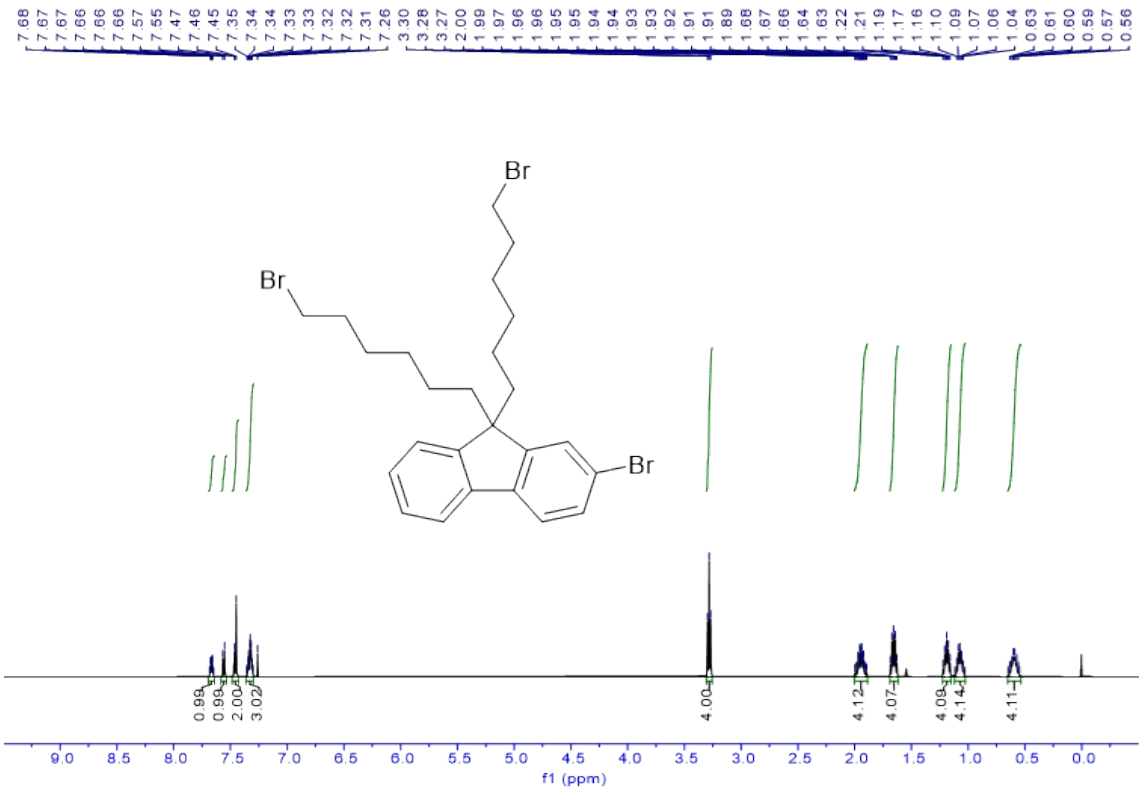
Scheme S3. **Synthesis of IR-FH3P and IR-FH4P.**



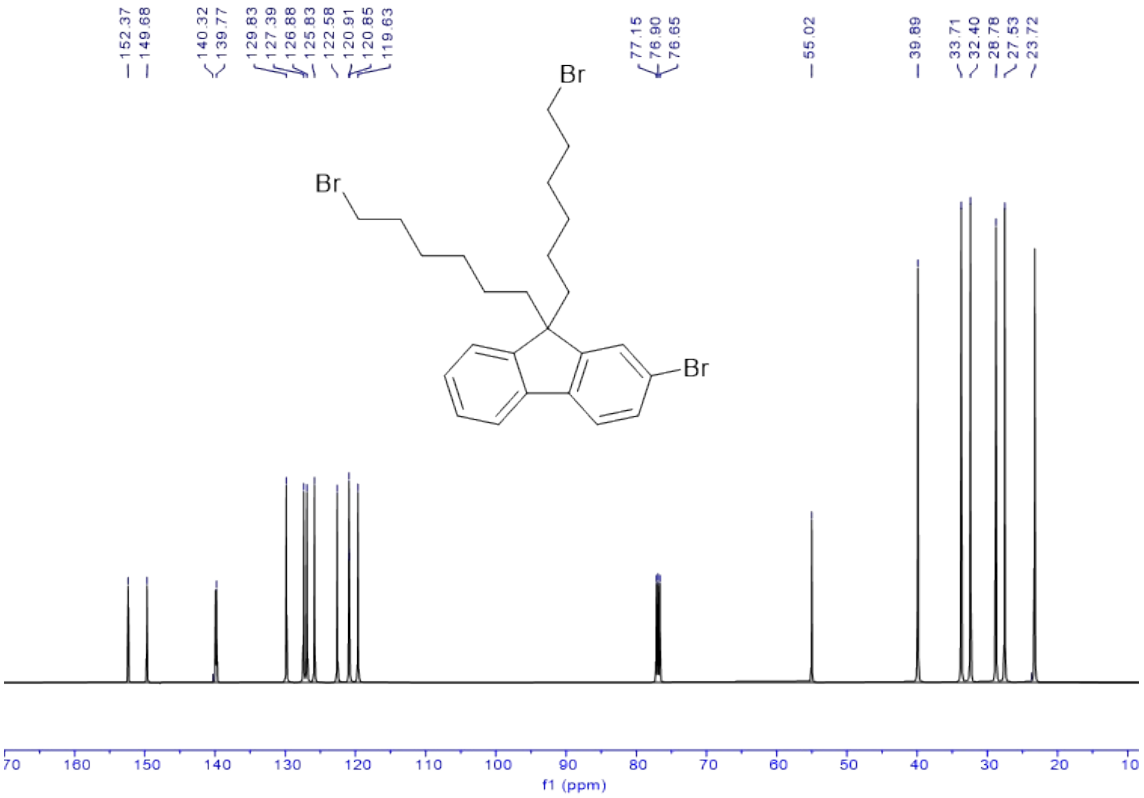
In a solution of IR-FH4 and IR-FH3 in DMF (15 mg, 0.1 M), NaN_3 (3 mg) was added under N_2 as a protection gas atmosphere. After reacting at 90 °C overnight, the product was extracted three times with 5 mL ethyl acetate and 5 mL brine. The combined organic phase was dried with anhydrous $MgSO_4$ and evaporated in vacuo. DMF was removed from the organic phase in vacuum oven under 60 °C without light**.** Without further purification, the compound was added into THF and mixed with alkene-PEG_{2000} (10 equiv.). Copper (I) thiophene-2-carboxylate (CuTc) (6 mg), and tris[(1-benzyl-1H-1,2,3-triazole-4-yl) methyl] amine (TBTA) (3 mg) were added into the solution. After stirring at 40 °C for 8 h, the mixture was poured into a 50 mL centrifuge tube and mixed with cold hexane. The cyan-colored compound was collected after 3 times washing by cold THF and Hex (10:1) solution. The compound was further purified by dialysis bag (3000 Da), then lyophilized to get dark green oil (IR-FH4P) and cyan oil (IR-FH3P). IR-FH4P and IR-FH3P were measured by SEC with 5500 *Mw* and 4980 *Mn*, respectively.

Scheme S4. NMR spectra of the synthesized compounds.

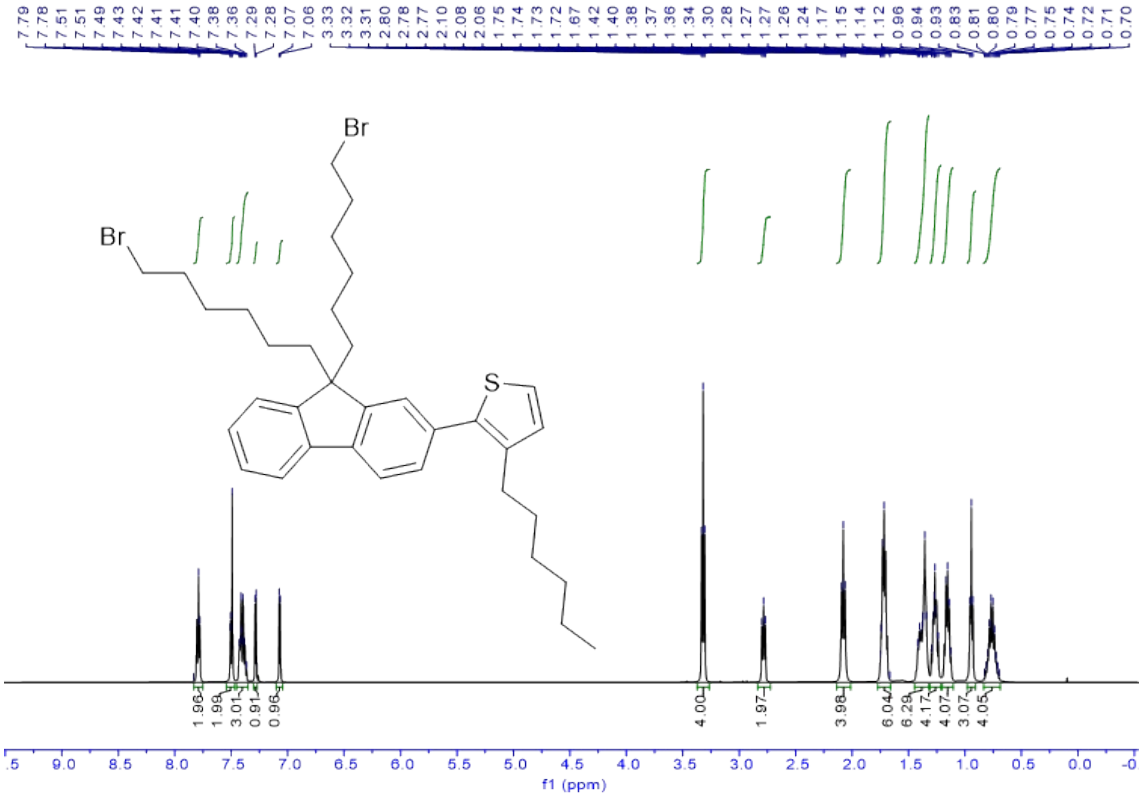
^{1}H NMR of **compound 2**



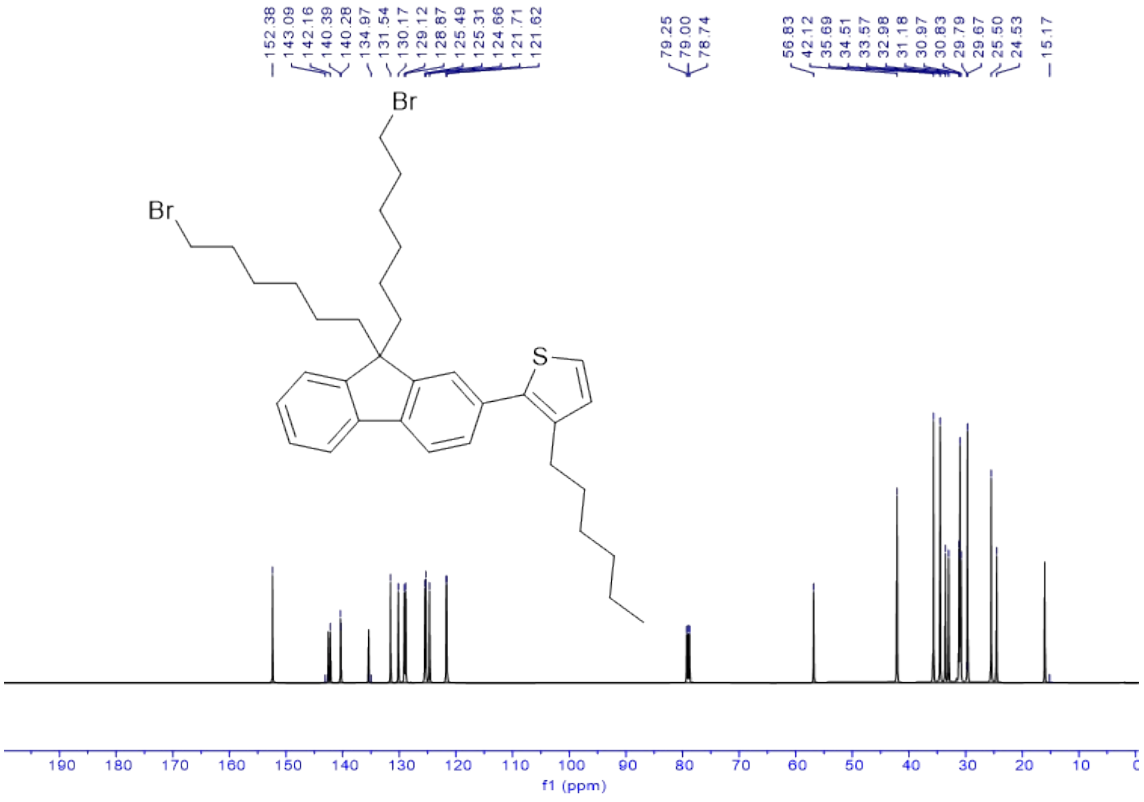
^{13}C NMR of **compound 2**



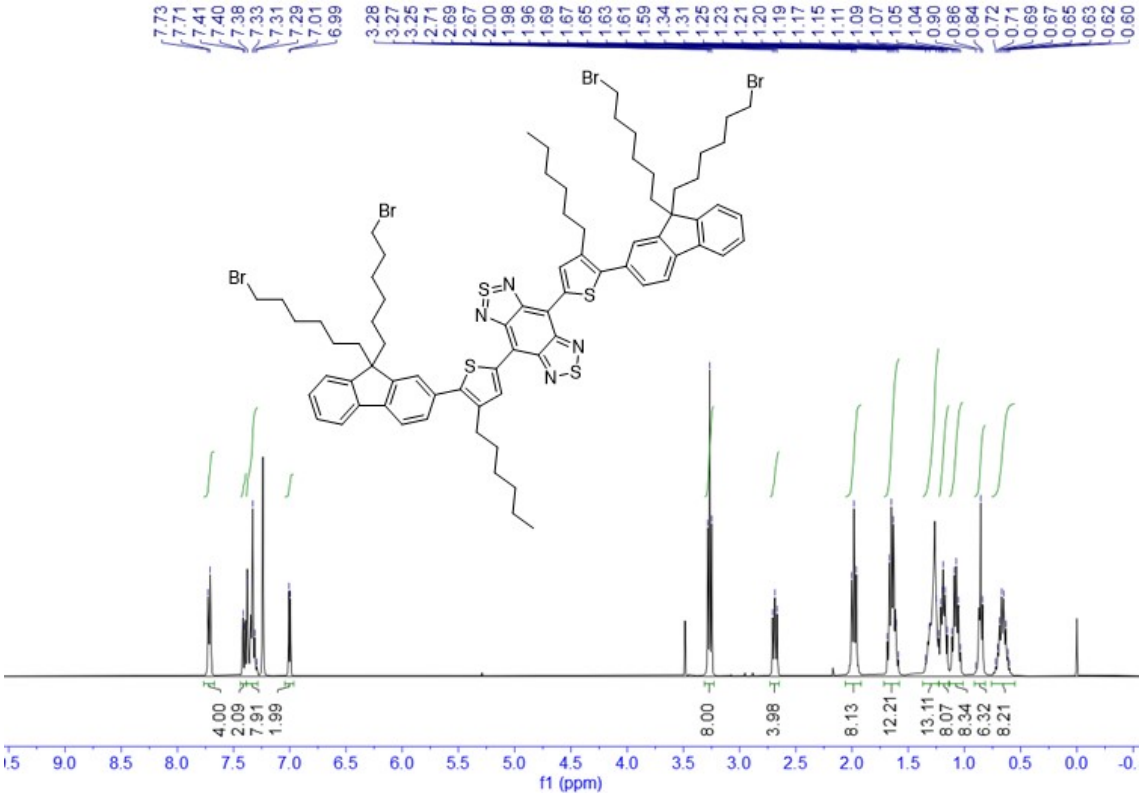
^{1}H NMR of **compound 3**



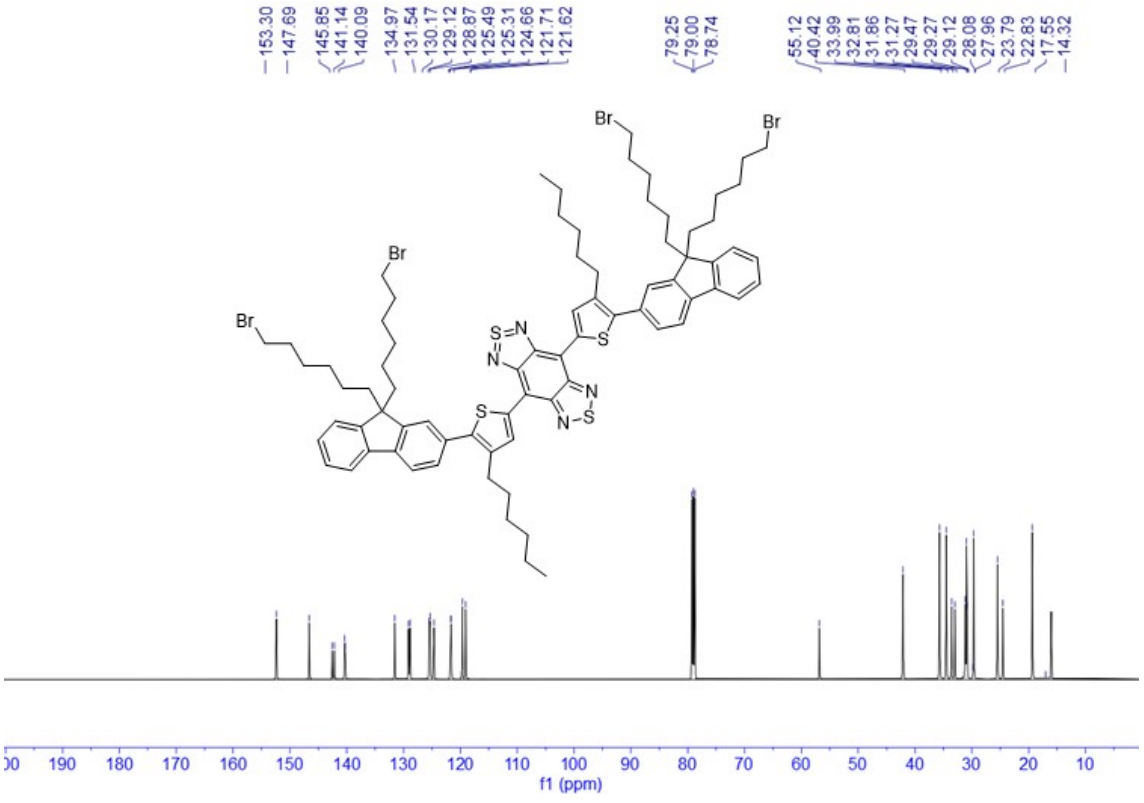
^{13}C NMR of **compound 3**



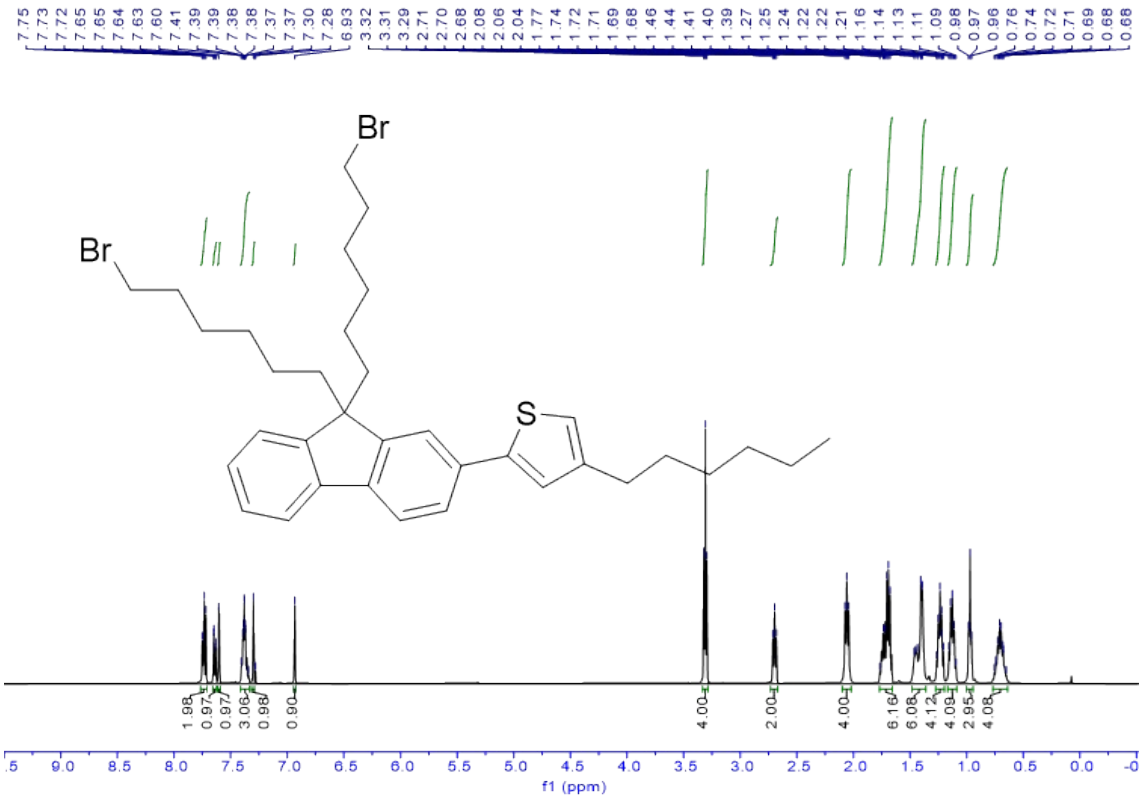
^{1}H NMR of **IR-FH4**



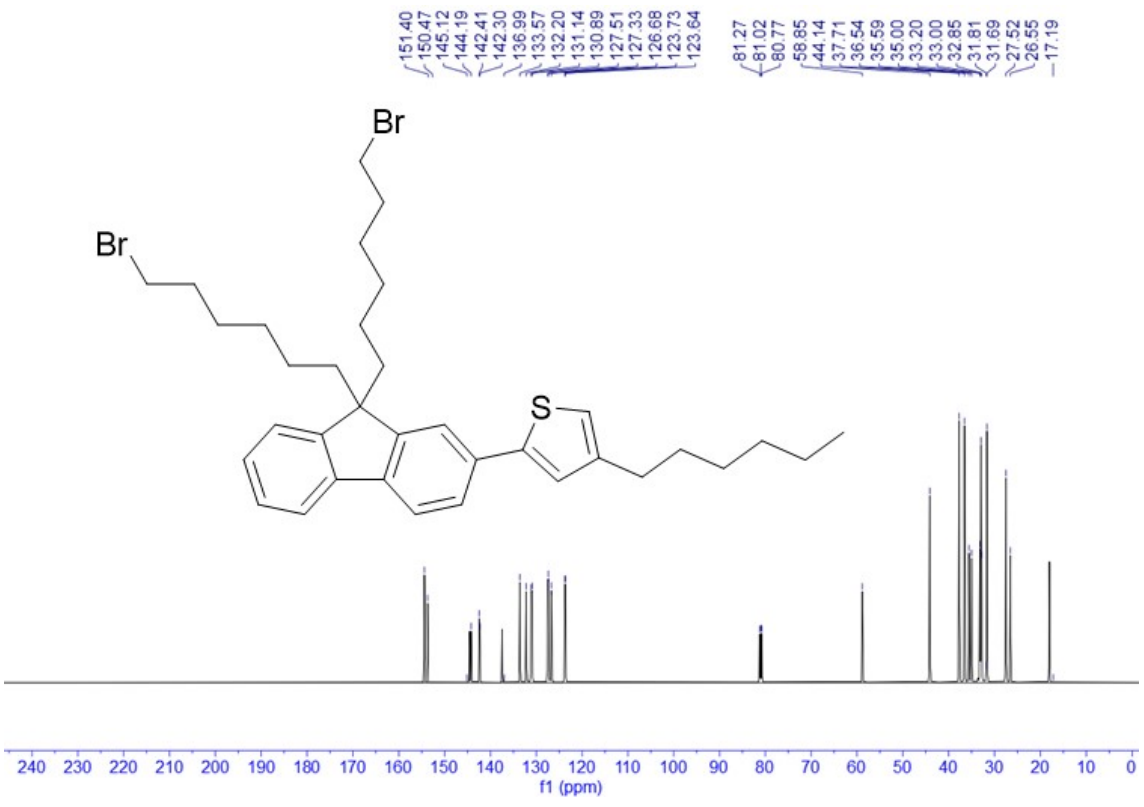
^{13}C NMR of **IR-FH4**



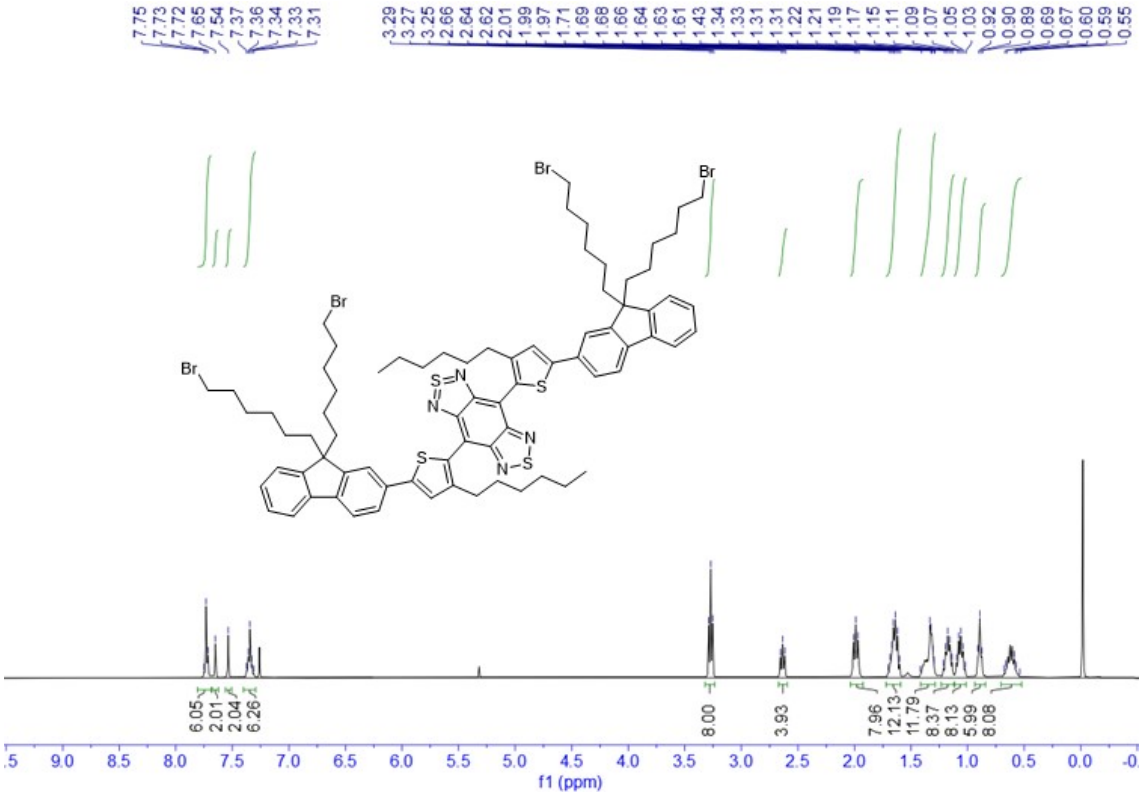
^{1}H NMR of **compound 5**



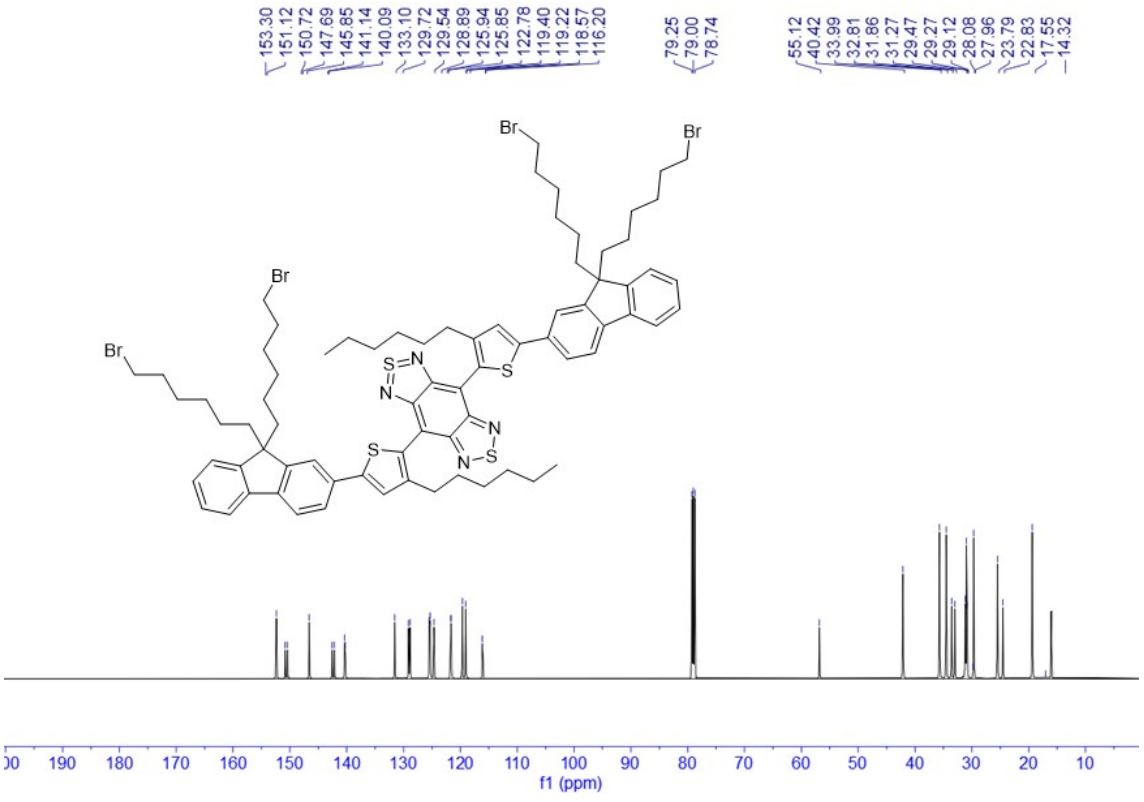
^{13}C NMR of **compound 5**



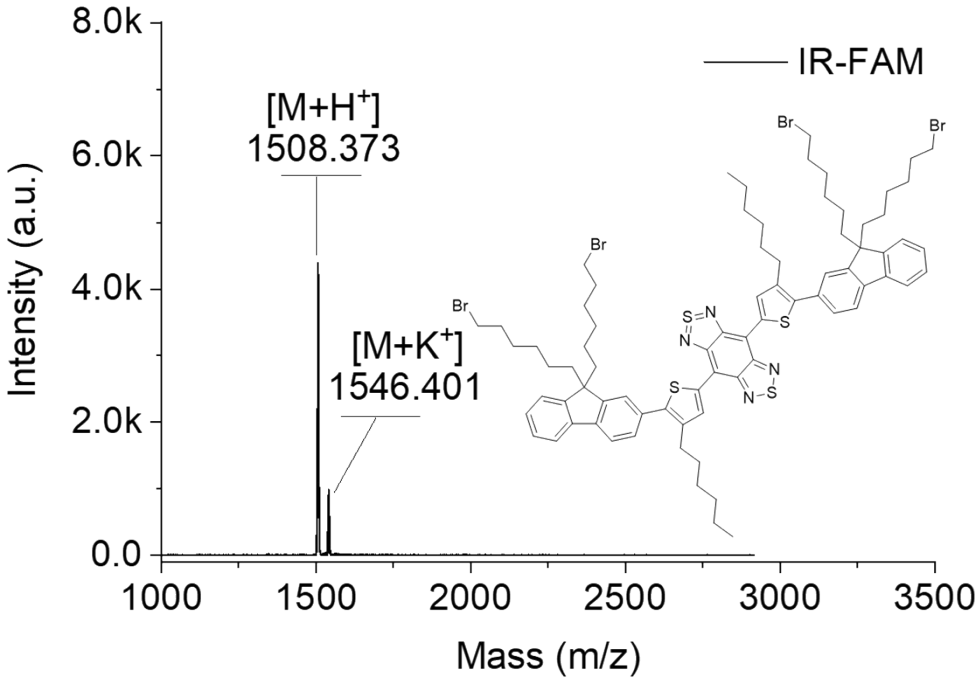
^{1}H NMR of **IR-FH3**

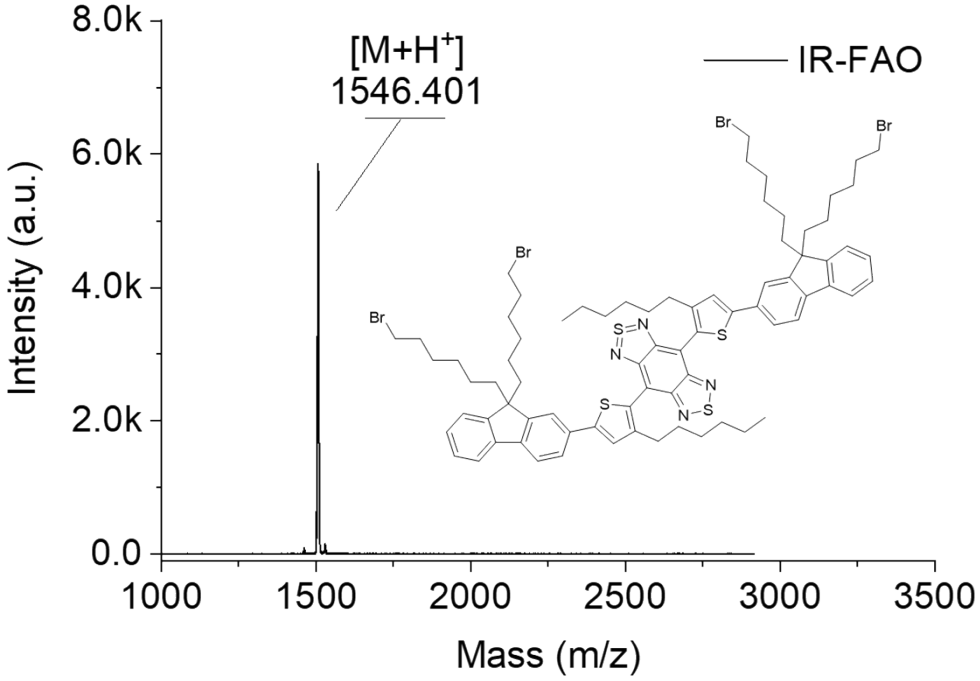


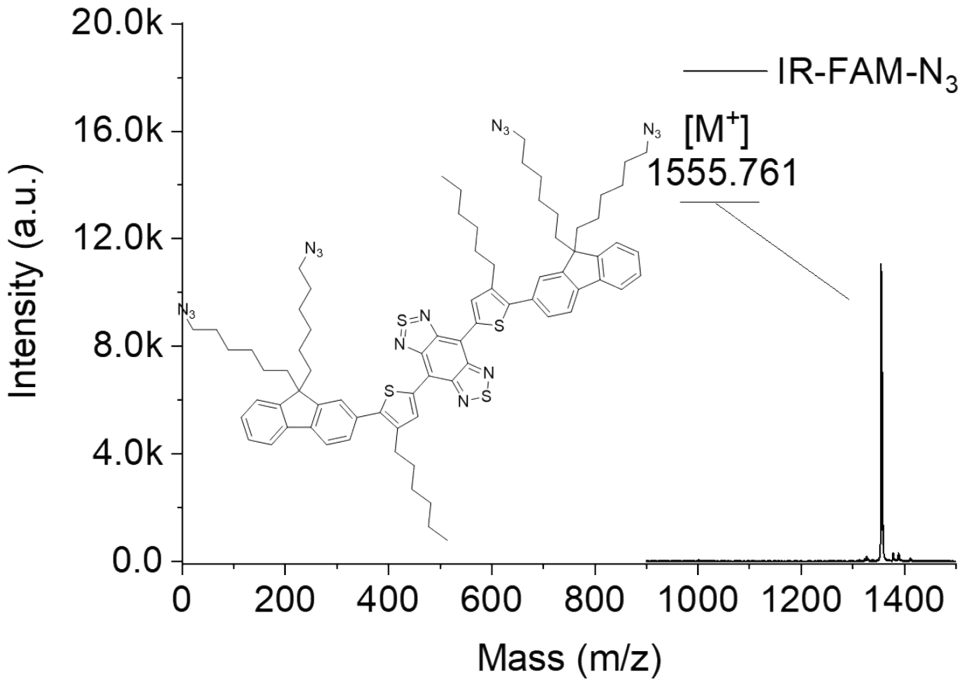
^{13}C NMR of **IR-FH3**

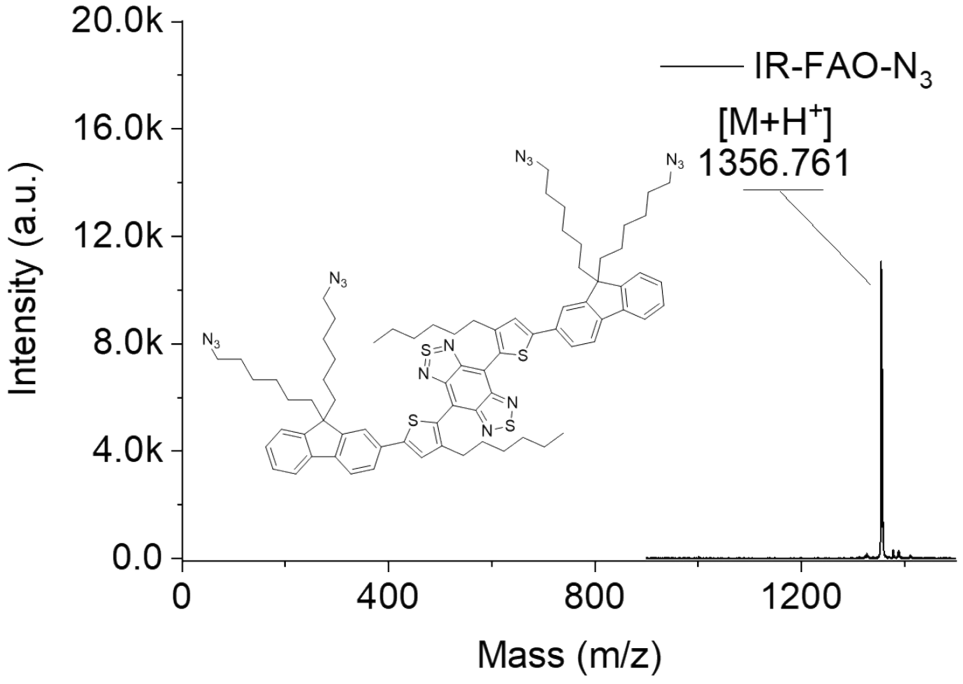


Scheme S5. MS spectra of the synthesized compounds






IR-FAM-N3
[M+]
1555.761
Intensity (a.u.)
Mass (m/z)
20.0k
16.0k
12.0k
8.0k
4.0k
0.0
0
200
400
600
800
1000
1200
1400


IR-FAO-N3
[M+H+]
1356.761
Intensity (a.u.)
Mass (m/z)
20.0k
16.0k
12.0k
8.0k
4.0k
0.0
0
400
800
1200

3. Supplementary figures

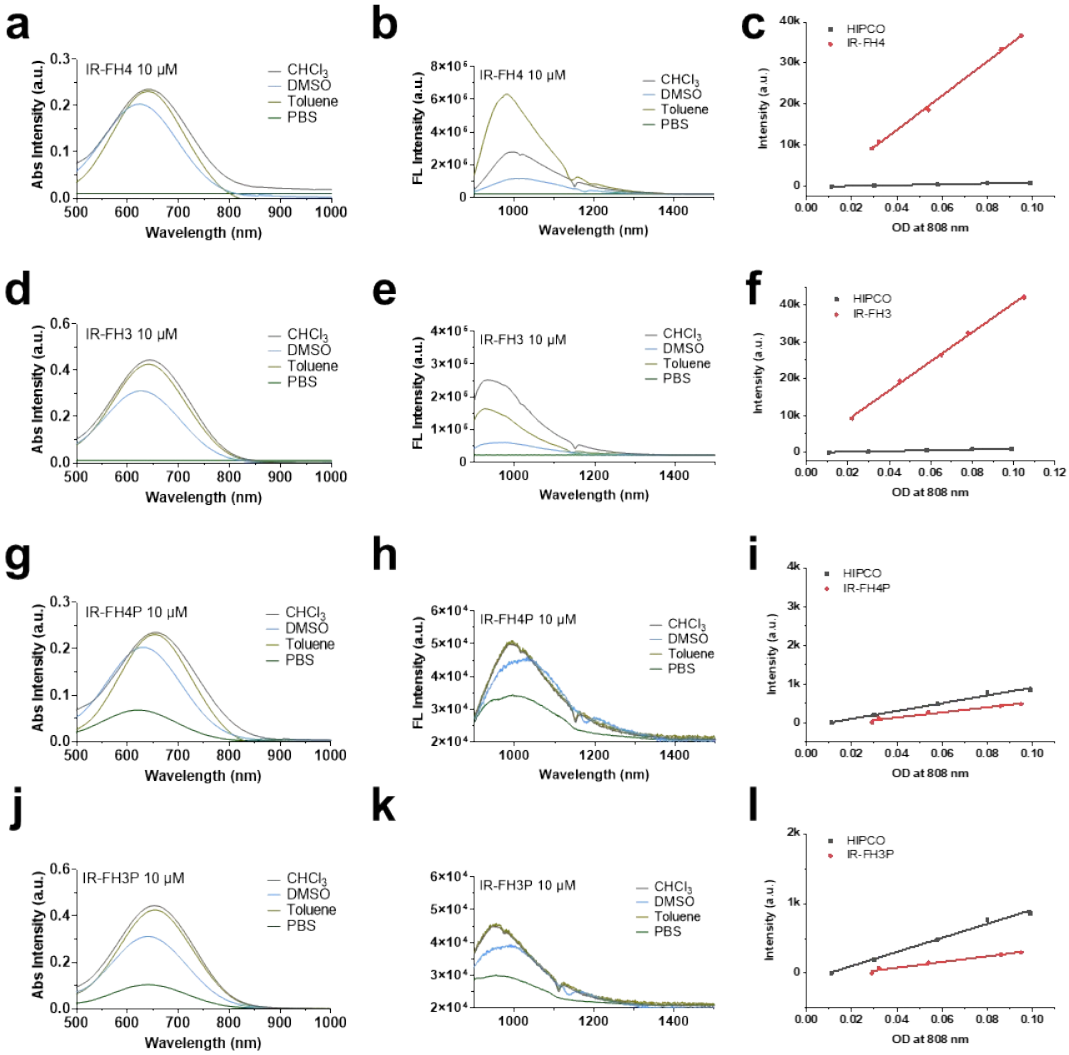


Figure S1. The absorption spectrum of **a)** IR-FH4, **d)** IR-FH3, **g)** IR-FH4P and **j)** IR-FH3P. NIR fluorescence spectrum of **b)** IR-FH4, **e)** IR-FH3, **h)** IR-FH4P and **k)** IR-FH3P. The integrated intensities of the emissions of the above samples were plotted against the absorbance at 808 nm for **c)** IR-FH4, **f)** IR-FH3, **i)** IR-FH4P and **l)** IR-FH3P.

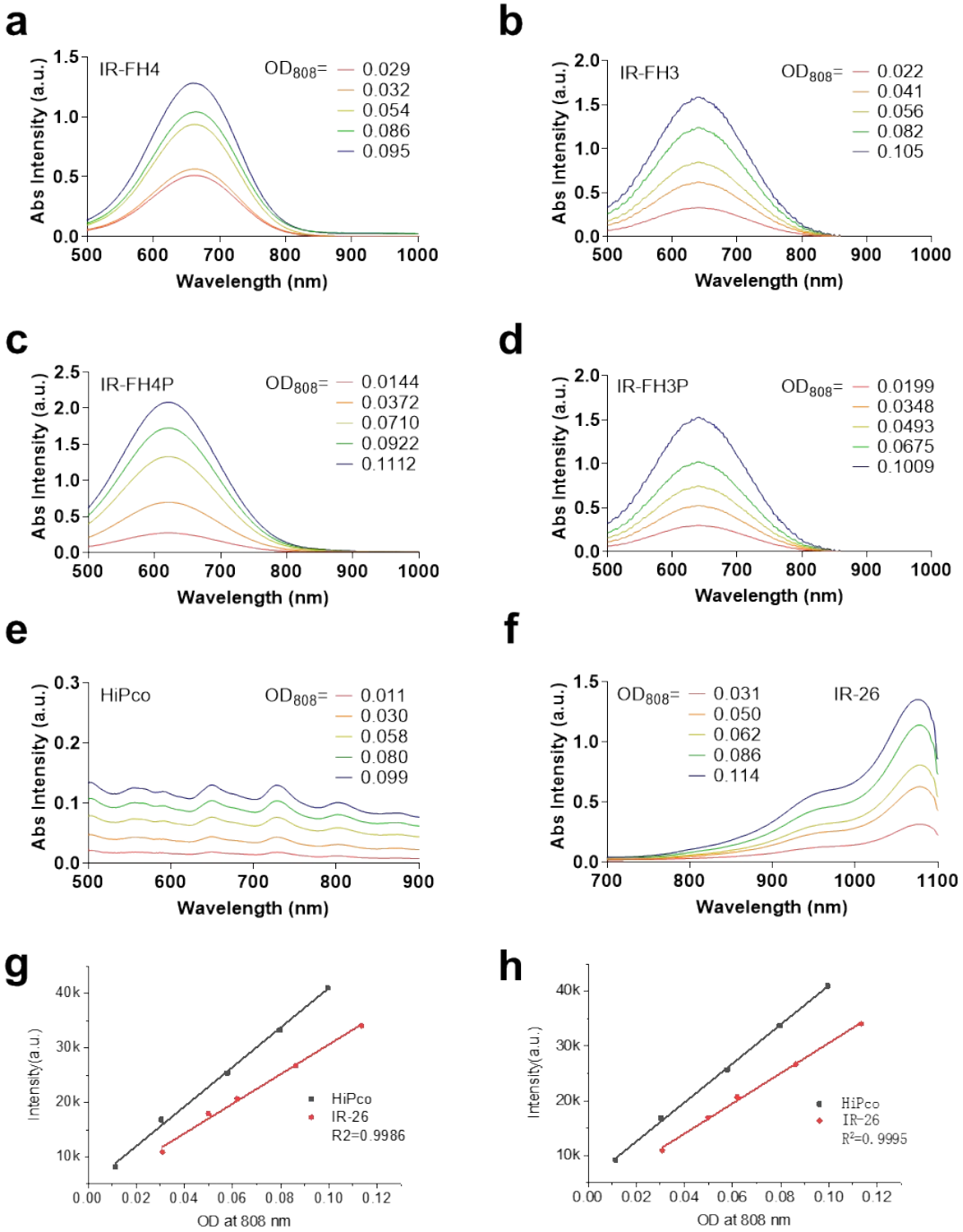


Figure S2. The absorption spectrum of various concentrations of **a)** IR-FH4, **b)** IR-FH3, **c)** IR-FH4P, **d)** IR-FH3P, **e)** SWCNTs and **f)** IR-26 ($OD_{808\ nm}$ =0.02-0.1). **g)** Quantum yield comparison between IR-26 and SWCNTs from InGaAs camera (808 nm excitation, 900 nm LP filter). **h)** Quantum yield comparison between IR-26 and SWCNTs obtained from fluorescence spectrometer (Edinburgh Instruments FL 920).

Data Note:

HiPco SWCNTs have been widely used as a reference for NIR-II quantum yield measurement.[1] Although the HiPco SWCNTs possess advantages in terms of stable fluorescence and easy preservation, the quantum yield was found variably from batch to batch. Therefore, we applied IR-26 as a second reference to correct the QY value of HiPco SWCNTs. After repeating the correction process multiple times, the QY of SWCNTs used in this paper was determined to be 0.06-0.55 % (IR26 = 0.05-0.5 %) at 900-1500 nm.

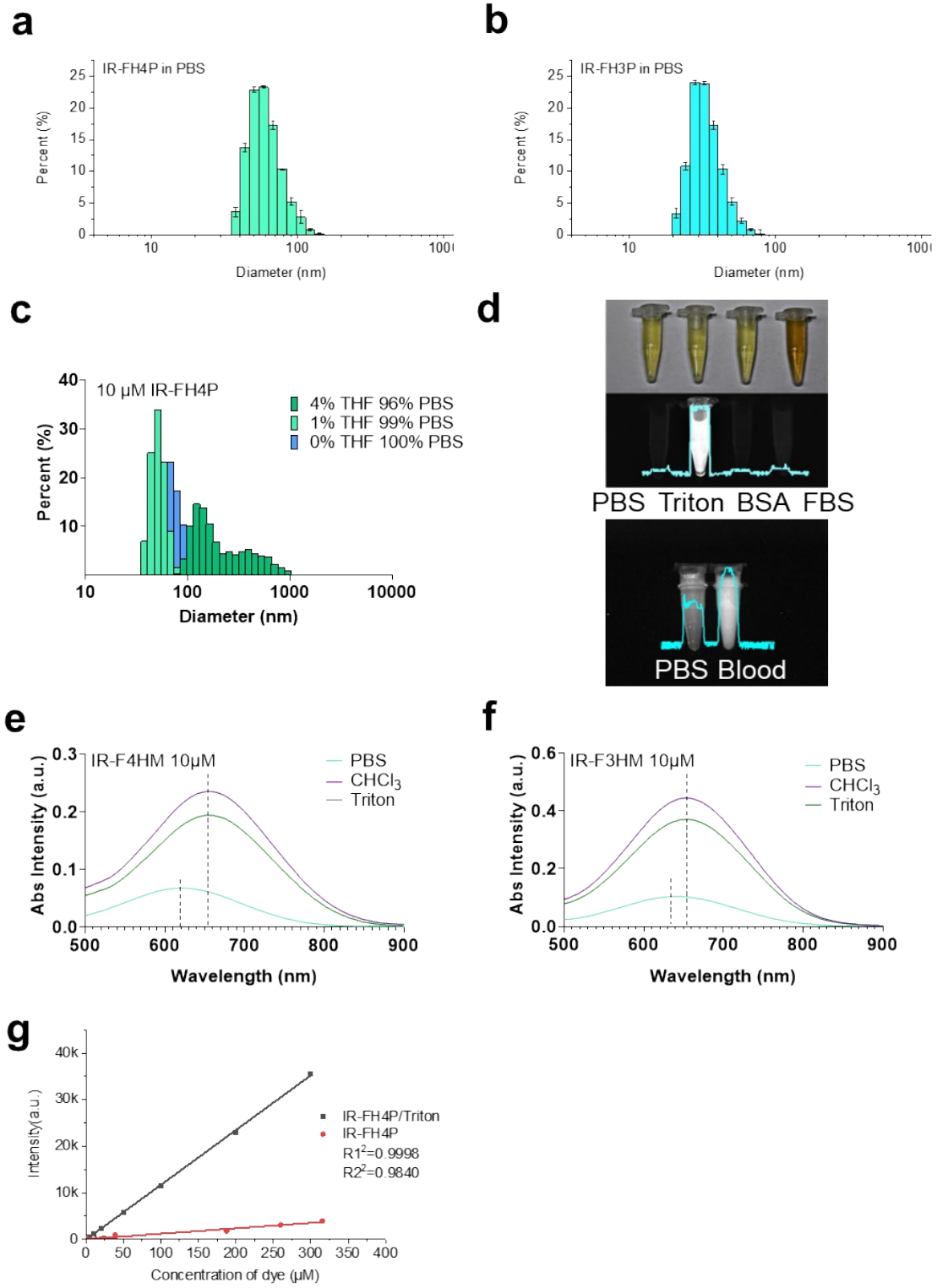


Figure S3: DLS of a) IR-FH4P (10 μM) and b) IR-FH3P (10 μM) in PBS. c) DLS of IR-FH4P (10 μM) in THF and THF/PBS mixture solutions. d) Photograph and NIR-II imaging of 100 μM IR-FH4P in PBS, Triton-X100, BSA, and FBS respectively, and NIR-II imaging of 100 μM IR-FH4P diluted into PBS and mice blood (10 μM). The absorption spectra (e, f) of IR-FH4P and IR-FH3P (10 μM in PBS buffer, $CHCl_3$, and 0.1% Triton-X100). g) Normalized

fluorescence intensity of IR-FH4P and IR-FH4P/Triton-X100 excited at 808 nm at different concentrations.

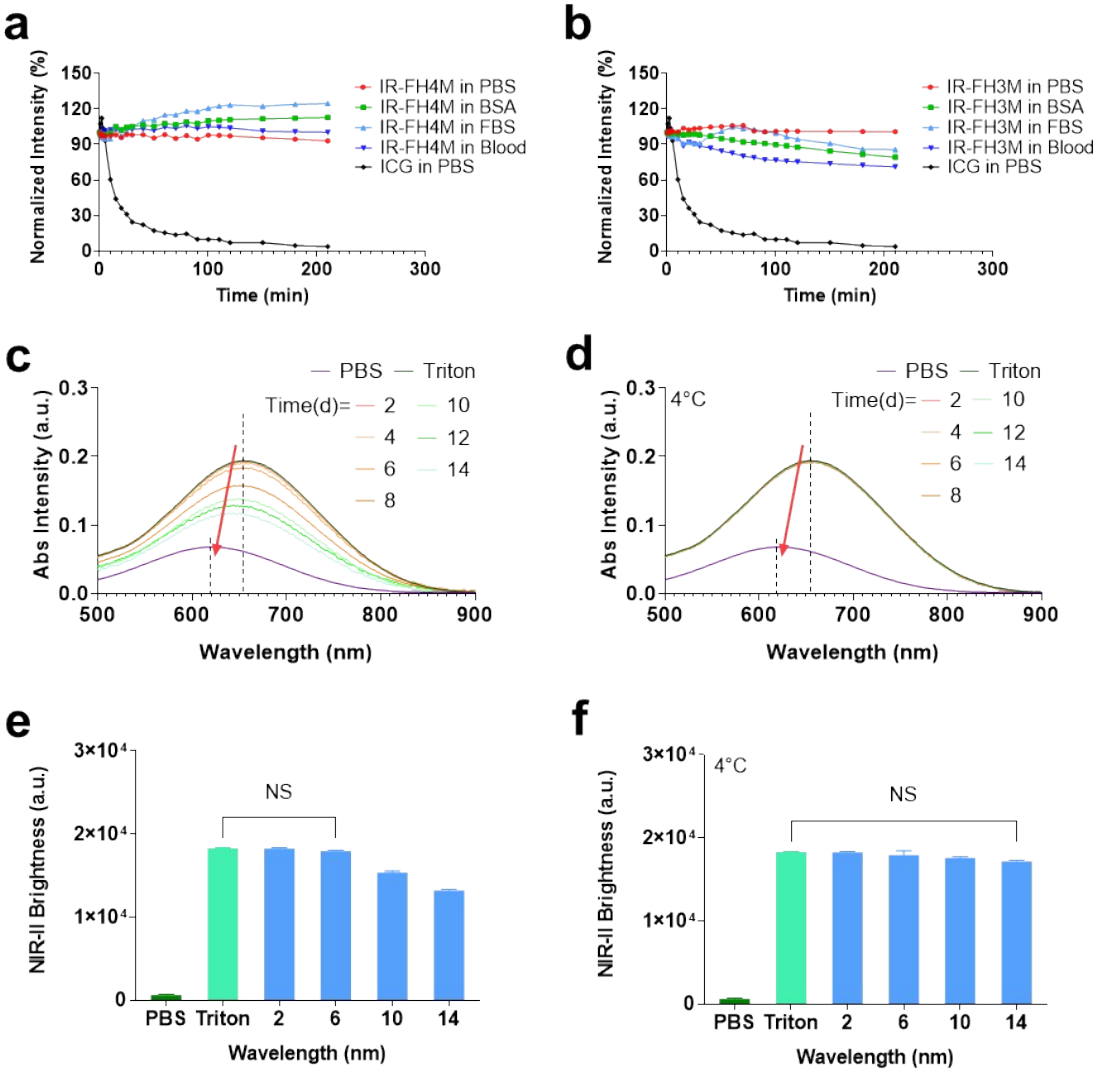


Figure S4. Photostability of **a)** IR-FH4P (10 μM) and **b)** IR-FH4P/Triton. The stability of IR-FH4P/Triton was measured through absorption spectra under c) R.T. condition and d) 4°C condition. FL intensity of IR-FH4P/Triton under e) R.T. condition and f) 4°C condition.

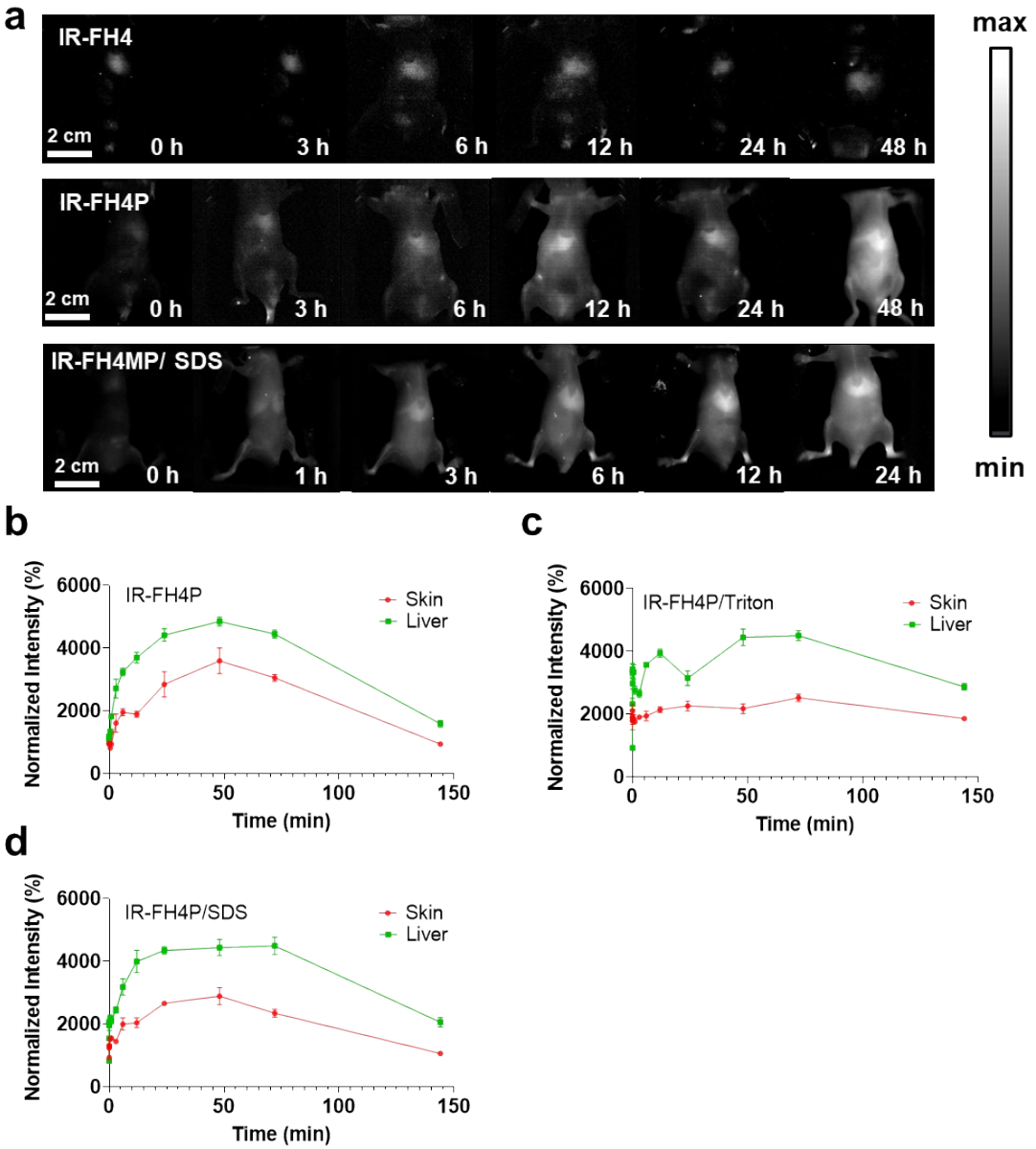


Figure S5. **a)** Selected time points from NIR-II imaging of mice in the supine position after intravenous injection of IR-FH4 (1% DMSO) (100 µL dye, OD = 0.5 at 808 nm), IR-FH4P (100 µL dye, OD = 0.5 at 808 nm), IR-FH4P/Triton-X100 (100 µL dye, OD = 0.5 at 808 nm, 10 µM surfactant) and IR-FH4P/SDS (100 µL dye, OD = 0.5 at 808 nm, 10 µM surfactant). Imaging conditions: 808 nm excitation, power density = 88 mW/cm^2, 900/1100 nm long pass filters). **b, c, d)** Liver signal of cohorts after IR-FH4P, IR-FH4P/Triton-X100, IR-FH4P/SDS administration.

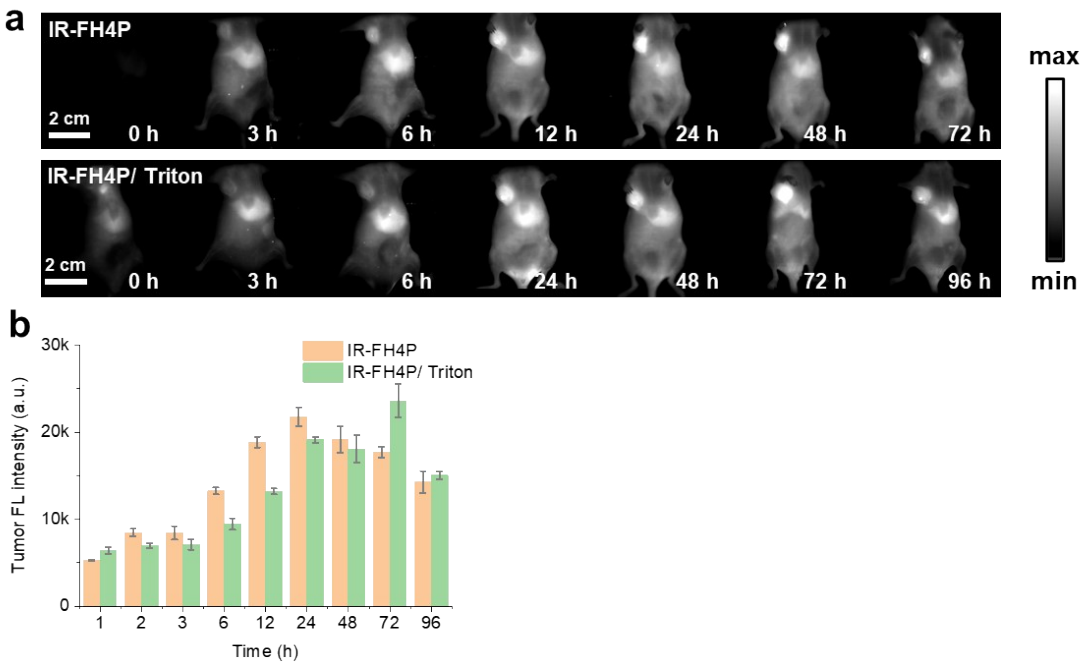


Figure S6. a) *In vivo* fluorescence imaging of 4T1 tumor-bearing mouse by IR-FH4P (100 μM) and IR-FH4P/Triton-X100 (100 μM, 10 μM surfactant). **b**) Fluorescence intensity of tumor at different post-injection time points.

Supplementary Tables

Table S1. QYs of IR-FH4, IR-FH3, IR-FH4P, and IR-FH3P in different solvents (HiPco SWCNTs were used as a reference with QY value 0.06-0.55%).

Dye	Solvent	λ_{abs}/ λ_{em}	Stokes shift	QYs (%)
IR-FH4	**$CHCl_3$**	**642/ 988**	**346**	**2.61-26.11**
	DMSO	**626/ 1002**	**376**	**0.80-8.04**
	Toluene	**638/ 981**	**343**	**2.56-25.63**
IR-FH3	**$CHCl_3$**	**642/ 940**	**298**	**2.46-24.63**
	DMSO	**626/ 948**	**322**	**0.95-9.47**
	Toluene	**638/ 941**	**303**	**2.32-23.16**
IR-FH4P	**$CHCl_3$**	**654/ 995**	**341**	**0.81-8.14**
	DMSO	**632/ 1012**	**380**	**0.73-7.25**
	Toluene	**650/ 998**	**348**	**0.83-8.29**
	PBS	**642/ 1016**	**374**	**0.04-0.36**
IR-FH3P	**$CHCl_3$**	**654/ 948**	**294**	**0.84-8.43**
	DMSO	**640/ 989**	**349**	**0.68-6.81**
	Toluene	**650/ 946**	**296**	**0.83-8.26**
	PBS	**640/ 965**	**325**	**0.02-0.21**

References

1. Q. Yang, Z. Hu, S. Zhu, R. Ma, H. Ma, Z. Ma, H. Wan, T. Zhu, Z. Jiang, W. Liu, L. Jiao, H. Sun, Y. Liang and H. Dai, *J. Am. Chem. Soc.*, 2018, **140**, 1715-1724.
2. S. Zhu, S. Herraiz, J. Yue, M. Zhang, H. Wan, Q. Yang, Z. Ma, Y. Wang, J. He, A. L. Antaris, Y. Zhong, S. Diao, Y. Feng, Y. Zhou, K. Yu, G. Hong, Y. Liang, A. J. Hsueh and H. Dai, *Adv. Mater.*, 2018, **30**, e1705799.
3. S. Diao, J. L. Blackburn, G. Hong, A. L. Antaris, J. Chang, J. Z. Wu, B. Zhang, K. Cheng, C. J. Kuo and H. Dai, *Angew. Chem. Int. Ed.*,2015, **54**, 14758–14762.